100. Eiler ME, Frohnmayer L, Larsen K, Owen J, eds. *Fanconi Anemia: Guidelines for Diagnosis and Management*. 3rd ed. Eugene, OR: Fanconi Anemia Research Fund, Inc.; 2008.
101. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev*. 2010;24(3):101–122.
102. Al-Rahawan MM, Giri N, Alter BP. Intensive immunosuppression therapy for aplastic anemia associated with dyskeratosis congenita. *Int J Hematol*. 2006;83(3):275–276.
103. Berthou C, Devergie A, D'Agay MF, et al. Late vascular complications after bone marrow transplantation for dyskeratosis congenita. *Br J Haematol*. 1991;79(2):335–336.
104. de la Fuente J, Dokal I. Dyskeratosis congenita: advances in the understanding of the telomerase defect and the role of stem cell transplantation. *Pediatr Transplant*. 2007;11(6):584–594.
105. Yabe M, Yabe H, Hattori K, et al. Fatal interstitial pulmonary disease in a patient with dyskeratosis congenita after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1997;19(4):389–392.
106. Dror Y, Freedman MH, Leaker M, et al. Low-intensity hematopoietic stem-cell transplantation across human leucocyte antigen barriers in dyskeratosis congenita. *Bone Marrow Transplant*. 2003;31(10):847–850.
107. Brazzola P, Duval M, Fournet JC, et al. Fatal diffuse capillaritis after hematopoietic stem-cell transplantation for dyskeratosis congenita despite low-intensity conditioning regimen. *Bone Marrow Transplant*. 2005;36(12):1103–1105, author reply 1105.
108. Ostronoff F, Ostronoff M, Calixto R, et al. Fludarabine, cyclophosphamide, and antithymocyte globulin for a patient with dyskeratosis congenita and severe bone marrow failure. *Biol Blood Marrow Transplant*. 2007;13(3):366–368.
109. Vuong LG, Hemmati PG, Neuburger S, et al. Reduced-intensity conditioning using fludarabine and antithymocyte globulin alone allows stable engraftment in a patient with dyskeratosis congenita. *Acta Haematol*. 2010;124(4):200–203.
110. Dietz AC, Orchard PJ, Baker KS, et al. Disease-specific hematopoietic cell transplantation: nonmyeloablative conditioning regimen for dyskeratosis congenita. *Bone Marrow Transplant*. 2011;46(1):98–104.
111. Nishio N, Takahashi Y, Ohashi H, et al. Reduced-intensity conditioning for alternative donor hematopoietic stem cell transplantation in patients with dyskeratosis congenita. *Pediatr Transplant*. 2011;15(2):161–166.
112. Fogarty PF, Yamaguchi H, Wiestner A, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. *Lancet*. 2003;362(9396):1628–1630.
113. Denny CC, Wilfond BS, Peters JA, Giri N, Alter BP. All in the family: disclosure of "unwanted" information to an adolescent to benefit a relative. *Am J Med Genet A*. 2008;146A(21):2719–2724.
114. Diamond LK, Shahidi NT. Treatment of aplastic anemia in children. *Semin Hematol*. 1967;4(3):278–288.
115. Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. *Clin Ther*. 2001;23(9):1355–1390.
116. Khincha P, Wentzensen I, Giri N, Alter BP, Savage SA. Response to androgen therapy and side effects in patients with dyskeratosis congenita. *Br J Haematol*. 2014;165:349–357.
117. Giri N, Pitel PA, Green D, Alter BP. Splenic peliosis and rupture in patients with dyskeratosis congenita on androgens and granulocyte colony-stimulating factor. *Br J Haematol*. 2007;138(6):815–817.
118. Giri N, Lee R, Faro A, et al. Lung transplantation for pulmonary fibrosis in dyskeratosis congenita: case Report and systematic literature review. *BMC Blood Disord*. 2011;11:3.

CHAPTER THREE

Telomere Biology in Stem Cells and Reprogramming

Luis F.Z. Batista[*,†]

[*]Department of Medicine, Washington University School of Medicine, St. Louis, Missouri, USA
[†]Department of Developmental Biology, Washington University School of Medicine, St. Louis, Missouri, USA

Contents

1. Introduction 68
   1.1 A variety of stem cells throughout organismal development 68
   1.2 Telomeres cap chromosomal ends 69
2. Telomere Homeostasis in Stem Cells 69
   2.1 Telomerase activity in pluripotent stem cells 69
   2.2 Telomerase activity in adult multipotent cells 71
   2.3 Organismal consequences of telomere attrition in stem cells 74
   2.4 Noncanonical roles of telomerase in stem cell regulation 75
3. Cellular Reprogramming 76
   3.1 Somatic cell nuclear transfer and cell fusion 76
   3.2 Reprogramming through induced pluripotency 78
4. Conclusions 81
References 81

Abstract

Telomerase expression in humans is restricted to different populations of stem and progenitor cells, being silenced in most somatic tissues. Efficient telomere homeostasis is essential for embryonic and adult stem cell function and therefore essential for tissue homeostasis throughout organismal life. Accordingly, the mutations in telomerase culminate in reduced stem cell function both *in vivo* and *in vitro* and have been associated with tissue dysfunction in human patients. Despite the importance of telomerase for stem cell biology, the mechanisms behind telomerase regulation during development are still poorly understood, mostly due to difficulties in acquiring and maintaining pluripotent stem cell populations in culture. In this chapter, we will analyze recent developments in this field, including the importance of efficient telomere homeostasis in different stem cell types and the role of telomerase in different techniques used for cellular reprogramming.

1. INTRODUCTION

1.1. A variety of stem cells throughout organismal development

Stem cells are functionally defined by their ability to proliferate in the same state (self-renew) while being able to generate differentiated cell types in the developing and adult organism.[1] Typically, stem cells are classified by their developmental potential, ranging from totipotent, represented by cells that are able to generate the whole organism, including the extraembryonic trophoblast lineage (zygote and early blastomeres in mammals), to unipotent cells that are able to differentiate into just one particular cell type (such as spermatogonial stem cells). Mouse embryonic stem (ES) cells are derived from the inner cell mass (ICM) of the embryo and represent the pluripotent stem cell state, able to generate the entire organism except the trophoblast lineage.[2,3] In addition, self-renewing, pluripotent stem cells have been derived from the mouse embryo at a slightly later stage, when the ICM has become the epiblast (these cells are then called epiblast stem cells). However, due to ethical concerns and technical challenges, it was not until almost 20 years later that James Thomson and colleagues were able to successfully derive human ES cells from the ICM mass of human blastocysts.[4] The relative ease of culturing mouse and human ES cells *in vitro*, together with the promise that these cells hold for regenerative medicine, prompted an extensive molecular and biochemical characterization of the pluripotent state and provided several insights into organismal development (for detailed reviews on ES cell biology, please see Refs. 5–8). On the other hand, adult stem cells have proven to be harder to isolate and to study *in vitro*.[9] These cells are more restricted in their developmental potential than ES cells and are typically classified as multipotent cells, able to differentiate into all cell types of a particular cellular lineage, being responsible for the replenishing of specific tissues throughout organismal life. The study of the molecular control behind each of these different stem cell states is fundamental for the understanding of mammalian development and human disease. In fact, a growing body of evidence suggests that the number of stem cells in adult tissues must be under strict genetic control in order to avoid uncontrolled expansion or exhaustion of a particular tissue, which can lead to different human conditions, such as cancer and aging.[10,11] Efficient stem cell function is therefore essential for organismal fitness. In this chapter, we will focus on how telomerase activity and telomere homeostasis are controlled in different stem cell populations

and on the importance of this process for proper stem cell self-renewal, a process that ultimately maintains the stem cell pool for the life of an organism.

1.2. Telomeres cap chromosomal ends

Telomeres are the physical ends of eukaryotic chromosomes. In vertebrates, these sequences are composed of long stretches of TTAGGG repeats that can extend up to 15 kb in humans and 100 kb in rodents. The telomeric DNA is composed of a long double-stranded tract that ends in a short, single-stranded overhang. This single-stranded unit of the telomere invades the double-stranded sequence, giving rise to a lasso-like structure named "T-loop" that protects the telomere terminus from the DNA damage-repair machinery that would otherwise recognize telomeres as potential DNA double-strand breaks.[12] Providing further protection to chromosome ends, telomeres are also bound by shelterin, a large multisubunit protein complex that prevents the chromosome ends from being recognized as a DNA break and inhibits inappropriate recombination. The physiological relevance of efficient telomere protection is well demonstrated by the inhibition or deletion of specific shelterin components, where there is rapid telomere uncapping, which results in a local DNA damage response at chromosome ends, leading to robust activation of DNA damage pathways.[13–17] More recently, it was discovered that in addition to shelterin, telomeres in vertebrates are also bound by proteins belonging to the CST complex, indicating that proper telomere homeostasis might result from cooperation between shelterin and CST to keep the 3′ overhang telomeric DNA stable.[18,19]

2. TELOMERE HOMEOSTASIS IN STEM CELLS

2.1. Telomerase activity in pluripotent stem cells

Due to the inability of DNA polymerases to fully replicate chromosome ends at the lagging strand[20] at every cell division, there is a loss of up to 200 bp of telomeric DNA in mammalian cells.[21] This is especially relevant for ES cells, since these have a rapid cell cycle progression characterized by an abbreviated G1 phase of the cell cycle.[22,23] This fast cell cycle is a defining characteristic of pluripotent cells, and it is thought to be necessary for continual self-renewal and to resist differentiation,[24] where the forced

expression genes that regulate progression through the cell cycle induce fast and potent cellular differentiation.[25,26] To avoid the significant telomere shortening that would otherwise be associated with continuous cellular divisions, mammalian pluripotent cells have high activity levels of telomerase, the ribonucleoprotein (RNP) enzyme responsible for synthesizing telomeres.[27–29] The importance of telomerase activity for the homeostasis and proliferation of pluripotent cells is clear from experiments using telomerase-ablated ES cells, which showed progressive telomere shortening, genomic instability, aneuploidy, and telomere fusions that culminated with reduced growth rates.[30] The only cells that maintained proliferation were the ones that were able to elongate their telomeres using telomerase-independent mechanisms.[31–33] Interestingly, it has recently been shown that mouse ES cells show a unique mode of telomere maintenance that relies on the transient expression of ZSCAN4, which promotes a rapid telomere extension by telomere recombination.[34] Interestingly, while the developmental potency of mouse ES cells is known to deteriorate during long-term cell culture, increasing the frequency of Zscan4 activation in mouse ES cells restores and maintains their developmental potency in long-term cell culture.[35] Confirming the importance of telomerase for pluripotency potential, tetraploid embryo complementation experiments (the most stringent test for pluripotency) have shown that telomerase-deficient mESCs with short telomeres lose their ability to generate complete ESC pups.[36] On the other hand, the overexpression of telomerase enhanced self-renewal, improved resistance to apoptosis, and increased proliferation in mouse[37] and human[38] ES cells. Biochemically, telomerase is a large, multisubunit protein complex that, in addition to TERT, its reverse transcriptase component is composed of TERC, the telomerase RNA component used as a template for the transcriptase reaction; dyskerin (DKC1), a TERC-binding protein necessary for its stability; and TCAB1, necessary for telomerase translocation to Cajal bodies and holoenzyme assembly correction.[39–41] While TERC is expressed in several somatic tissues, TERT is only found in stem and progenitor cells, rendering the vast majority of human adult somatic cells telomerase negative.[42] The high levels of TERT in pluripotent cells culminate in telomere elongation during the derivation of both mouse[43] and human[44] ES cells from the ICM of blastocysts. In addition, both TERT expression and telomerase activity are rapidly downregulated during differentiation of pluripotent cells,[45,46] which indicates that they can be regarded as markers of undifferentiated ES cell populations.

2.2. Telomerase activity in adult multipotent cells

Most adult somatic tissues present a complex cellular hierarchy that starts with a small number of multipotent stem cells that can either self-renew (thereby maintaining the stem cell pool) or differentiate into progenitor cells that have a limited proliferative potential and usually differentiate into mature, functional, tissue somatic cells.[47] Adult stem cells are normally detected as long-lived cellular populations that reside in specialized niches of a given tissue and are maintained in a quiescent state. However, a growing body of evidence indicates the existence of long-lived, yet actively cycling, adult stem cell populations.[48–50] These cells would support the homeostasis of rapidly dividing organs, such as the gut and the skin, and the hematopoietic system. To be able to maintain self-renewal, while actively cycling to generate progenitor cells, these active adult stem cell compartments must retain efficient telomere maintenance mechanisms. Accordingly, despite difficulties in isolating and characterizing adult stem cells, telomerase activity has been detected in different adult stem cell populations. We will now analyze different adult stem cell compartments in more detail.

2.2.1 Hematopoietic system

Samples from the hematopoietic system are more directly obtainable than most other adult tissues, which translated to more detailed research on the functional and molecular characterization of its components. Telomerase activity was described in early experiments with the hematopoietic system, including in hematopoietic stem cells that give rise to all other cells in the blood.[51,52] In fact, elegant experiments from the Weissman group demonstrated that the frequency of telomerase-expressing cells within different populations of the hematopoietic system was proportional to the frequency of cells thought to have self-renewal potential. Among bone marrow hematopoietic stem cells, 70% exhibited detectable telomerase activity.[51] However, telomeres in hematopoietic cells, including hematopoietic stem cells (HSCs), shorten during organismal aging in both mice[53] and humans,[54] which indicates that the amount of telomerase activity is insufficient to cope with the constant renewal that occurs in the blood system. The importance of telomerase for the long-term replicative capacity of HSCs was first exemplified in serial transplantation experiments, where telomerase-deficient HSCs could be serially transplanted for only two rounds, whereas wild-type HSCs could be serially transplanted for at least four rounds. During these experiments, the rate of telomere shortening was increased approximately

twofold during serial transplantation of telomerase-deficient HSCs.[55] Interestingly, HSCs overexpressing telomerase did not show an increased repopulation capacity, showing that telomere-independent barriers may act independently and limit the transplantation capacity of HSCs.[56]

2.2.2 Hair follicle stem cells

Telomerase activity has also been identified in mitotically active segments of the hair follicle, which later were discovered to be the niche for hair follicle stem cells.[57] Interestingly, telomerase activity was shown to be higher in ultraviolet light-damaged skin, which can indicate either that telomerase is involved in the early stage of skin carcinogenesis or that telomerase helps tissue regeneration.[58] In fact, using telomere shortening inhibited the mobilization of stem cells out of their niche, impaired hair growth, and resulted in the suppression of the regenerative capacity of these cells *in vitro*.[59] On the other hand, induction of TERT in mouse skin epithelium caused a rapid activation of the hair follicle cycle (from telogen, the resting phase, to anagen, the active phase) culminating in robust hair growth.[60]

2.2.3 Intestinal stem cells

The constant mechanical damage inflicted on the gut by passing bowel content, combined with the chemical and biological assault by the luminal contents, leads to a nearly complete renewal of the intestinal epithelium every 4–5 days, therefore constituting the fastest self-renewing tissue of the human body.[61,62] This fantastic rate of self-renewal, together with a very distinct organizational anatomy, transformed the intestine into one of the most studied models in the adult stem cell field. In a tissue with such a constant need for renewal, it is not surprising that telomerase activity was identified in intestinal stem cells.[63–65] Interestingly, while the Breault group used a GFP-TERT transgenic mouse-model approach to conclude that TERT-positive stem cells in the gut are Bmi1-positive and LGR5-negative and represent a rare and slow-cycling stem cell population,[63] Hans Clevers group used a more sensitive technique, where they would sort LGR5-positive cells and analyze telomerase activity by TRAP (telomere repeat amplification protocol) to conclude that the fast-cycling LGR5-positive cells in the gut actually have high telomerase activity.[65,66]

2.2.4 Muscle stem cells

Recently, investigators from Stanford University were able to demonstrate the importance of telomerase activity also for muscle homeostasis. Working with a Duchenne muscular dystrophy mouse model (mdx) lacking the RNA component of telomerase (TERC), the authors show that telomere attrition causes severe muscular dystrophy that progressively worsens with age.[67] The worsening of symptoms was due to the loss of adult muscle stem cells and could be ameliorated histologically by the transplantation of wild-type muscle stem cells in telomerase-deficient mice. These results not only demonstrate the importance of telomere in muscle stem cell homeostasis but also indicate that Duchenne muscular dystrophy is a stem cell disease. Moreover, it was also demonstrated that telomere dysfunction plays a role in cardiac failure in Duchenne muscular dystrophy, where demands of contraction in the absence of dystrophin, coupled with increased oxidative stress in these settings, cause accelerated telomere erosion, which culminates in cardiac failure and death.[68]

2.2.5 Neural stem cells

The identification of neural stem cells (NSCs) has, historically, been challenging. However, several breakthroughs during the last years collectively indicated that the subventricular zone (SVZ) and the subgranular zone of the hippocampus represent neurogenic niches or local microenvironments that permit and support neurogenesis.[69] Because telomerase expression is so closely related to stem cell function, different groups have tried to identify telomerase-positive cells in the brain. Using *sox2* as a prospective marker for NSCs, it was recently shown that sox2-positive cells isolated from the SVZ were capable of sustained mitotic expansion and showed high telomerase activity.[70] Additionally, it was also shown that telomere attrition impaired the proliferation of adult NSCs that were isolated from the SVZ of telomerase-ablated mice. Curiously, the same authors reported that telomere attrition did not affect the *in vivo* proliferation potential of embryonic NSCs.[71] Thus, despite technical limitations that still complicate the study of adult neurogenesis, it seems reasonable to assume that at least, a percentage of NSCs are telomerase-positive and that telomere homeostasis is important for the maintenance of the adult stem cell pool in the brain. Certainly, with higher definition (deeper and to a higher resolution) of *in vivo* imaging techniques, we will be able to track telomerase-positive cells and understand their precise role in neurogenesis.

2.2.6 Spermatogonial stem cells

Throughout an individual's life, the spermatogenic process relies on proper regulation of self-renewal and differentiation of the spermatogonial stem

cells. These are rare single cells that comprise about only 0.03% of the total number of germ cells and are situated on the basal membrane of the seminiferous epithelium. Interestingly, telomere length seems to elongate over the human life span, and this is presumed to be due to high telomerase activity in spermatogonial stem cells throughout the life of a male.[72] In fact, although the knowledge about the identity and characteristics of spermatogonial stem cells in human is very limited, initial experiments with adult testes from Rhesus monkeys identified a population, localized at the basement membrane of seminiferous tubules, that showed high telomerase activity.[73] These results have recently been corroborated in spermatogonial stem cells from adult human testis.[74] The presence of telomerase in the human male germ line seems to be functionally relevant, since the presence of single-nucleotide polymorphisms in TERT and in TEP1 (telomerase-associated protein 1) was recently associated with susceptibility to male infertility.[75]

Interestingly, telomerase activity is low or even absent in oocytes and cleavage-stage embryos, and it is only recovered in blastocysts.[42] Accordingly, telomere length in the oocyte is shorter than in somatic cells. The mechanisms responsible for telomere resetting during the early stages of development are fascinating. Telomeres lengthen considerably following activation of the egg (in mice, thousands of base pairs are added within the first two cell cycles). This happens despite low levels of telomerase activity and seems to be driven by extensive telomere sister-chromatid exchange, and accordingly, DNA recombination proteins were colocalized to telomeres in early cleavage-stage embryos.[76] Interestingly, following this early stage and just as telomerase activity increases in blastocysts, the proteins for recombination and DNA damage repair, as well as Telomere-Sister Chromatid Exchange (T-SCE), decrease markedly, suggesting that from this stage on, telomerase is responsible for telomere elongation, which is corroborated by experiments showing that telomere elongation at this stage of differentiation is abrogated in telomerase-deficient mice.[77] This fascinating dual mode of telomere elongation is most likely responsible for the telomere length resetting during the vertical transfer of genomic material through time.

2.3. Organismal consequences of telomere attrition in stem cells

The importance of telomerase activity in organismal fitness was first delineated from the generation and characterization of laboratory mice null for either TERT or TERC. Although early generation (G1) of these mice has a lack of phenotype, late generations (>G3) present short telomeres and an associated shortened life span, decreased fertility, and impaired organ function[78,79] that are more readily observable in tissues with high turnover rates.[80] More recently, reduced tissue renewal capability in high-turnover tissues was also observed in mouse models with mutant shelterin, providing a clear link between telomere dysfunction and tissue renewal in rodents.[81–84] In humans, the importance of proper telomerase function in adult stem cell compartments is readily observable in patients suffering from different syndromes associated with dysfunctional telomeres, such as the inherited bone marrow failure syndrome dyskeratosis congenita (DC).[85,86] To date, all known mutations in DC patients have been found in genes responsible for telomere homeostasis, and an underlying characteristic of this disease is the presence of short telomeres, usually below the first percentile when compared with the rest of the population.[87] Patients with DC present with systemic tissue defects that are more pronounced in organs with high turnover rates, suggesting an exhaustion of actively cycling tissue stem cells due to a telomerase deficiency.[88] More details of human diseases associated with telomere dysfunction are described elsewhere in this book.

2.4. Noncanonical roles of telomerase in stem cell regulation

The experiments described above with germ-line telomerase knockout mice indicate that the deleterious effects of telomerase deficiency are only observed after a long time frame, with the continuous inheritance of progressively shortened telomeres from one generation to the other. However, recent evidence suggests that TERT is able to directly stimulate stem cells, in a mechanism independent from its telomere elongation function. Initial experiments using TERC knockout and TERT catalytically inactive mice showed that conditional induction of TERT in skin epithelium causes rapid activation of hair follicle stem cells present in the bulge region, inducing robust hair growth.[60] This noncanonical role of telomerase in stem cell regulation has been confirmed by different groups, both in mouse[59] and in zebra fish.[89] Analysis of genome-wide transcriptional response to acute changes in TERT levels in mouse skin revealed that the TERT transcriptional response closely correlates with the response mediated by both Myc and Wnt, two proteins that are intimately associated with stem cell function.[90] Corroborating this idea, it has been shown that the TERT noncanonical activity in stem cell activation is mediated by its interaction with Brg1 and β-catenin, the central activator of the canonical Wnt pathway.[91] Interestingly, it was also shown that β-catenin regulates TERT expression through an

with short telomeres. It is also unclear why Dolly died at an early age. Most likely, these differences are the consequence of improved protocols for SCNT cloning and probably intrinsic characteristics of donor cells. Nevertheless, it is now well accepted that telomerase is activated in cloned embryos that were derived from donor nuclei with undetectable levels of telomerase. Interestingly, also in cloned embryos, the activation of telomerase is again found at the blastocyst stage of embryogenesis.[116,117,120] Importantly, a recent study suggests that the telomere elongation in cloned embryos is independent of the type and telomere length of the donor cell and is intrinsic to the cloning process itself.[121]

3.2. Reprogramming through induced pluripotency

The successful cellular dedifferentiation using SCNT and cell-fusion strategies provided proof that mammalian development does not require irreversible changes in the genome. However, those techniques are cumbersome, and SCNT is controversial since it involves the use and destruction of human embryos. Therefore, it is not surprising that the discovery of an easy and fast method that is free of potential ethical issues for cellular reprogramming was received with great exhilaration by the regenerative medicine community. Decades of research in cellular reprogramming have recently culminated in the development of "induced pluripotent stem cells" (iPSCs).[122–124] With this technology, adult somatic cells can be reprogrammed back to a pluripotent state, by forced expression of transcription factors associated with the stem cell state. The most commonly used factors, Oct-4, Sox-2, Klf4, and c-Myc, have been used to reprogram cells from a growing number of species, using a variety of reprogramming methods, including RNA,[125] microRNA,[126] and protein transduction.[127] These cells have represented the biggest breakthrough in regenerative medicine in the last decades, and several groups are actively generating patient-specific iPS cells from a variety of diseases. The hope is that these cells will help in the understanding of developmental aspects of different syndromes and that they can be used as platforms for drug discovery and future transplantation sources (for a detailed review on the use of iPS cells for regenerative medicine, see Ref. 128). However, before these cells can be used in clinical settings, we need to confirm that they represent, in fact, a pluripotent state that is safe for human experimentation. Since telomerase activity and telomere attrition are so fundamental for stem cell biology, it comes as no surprise that a lot of emphases are given to understanding telomerase and telomere regulation in settings of induced reprogramming by defined factors.

3.2.1 *Telomere reprogramming in iPS cells*

Our ability to use induced pluripotent stem cells for cellular therapy requires that these cells have unrestricted proliferative capacity and that they maintain genome integrity during extensive periods in culture. Both of these requirements are directly associated with efficient telomerase activity and telomere homeostasis. In fact, consistent with a pluripotent state, initial reports of iPS cell generation showed that TERT was effectively upregulated after cellular reprogramming in both mouse[123] and human[122] cells. Moreover, reprogramming cells from a mouse expressing a GFP-TERT reporter, it was determined that the reactivation of TERT expression and telomerase activity was a late event during the cellular reprogramming process.[129] Moreover, the addition of TERT to the reprogramming "cocktail" (the four factors commonly used for iPS cell generation) increased reprogramming efficiency of human fibroblasts.[130] Confirming the importance of telomerase activity for cell reprogramming, it was shown that telomere elongation following cellular reprogramming is dependent on telomerase and not on alternative mechanisms of lengthening.[131] Likewise, the same authors show that although iPS cells were effectively generated from telomerase knockout mouse cells with long telomeres, the efficiency of reprogramming of telomerase-ablated cells with short telomeres is significantly reduced.[131] The low efficiency of cellular reprogramming in cells with short telomeres and other types of genetic damage can be rescued by abrogation of the p53 DNA damage response pathway, indicating that it could possibly work as a mechanism to ensure the genetic integrity of reprogrammed cells.[132–136] Giving further support for the importance of telomere homeostasis during cellular reprogramming, it has been shown that iPS cells with longer telomeres generate mouse chimeras with higher efficiency than those with short telomeres, when injected into blastocysts.[36] Interestingly, a recent paper compared the efficiency in cellular reprogramming of telomerase-ablated mouse cells, using either SCNT or induced pluripotency. Consistent with previous results described here earlier,[76] telomeres were elongated dramatically in cells reprogrammed using SCNT, due to a telomerase-independent mechanism. However, cells reprogrammed to an iPS state did not show telomere elongation during the process. This led to a higher differentiation potential and self-renewal capacity of cells generated by SCNT when compared to iPS cells.[137] Curiously, it has recently been

shown that overexpression of catalytically active TERT is able to prevent X-chromosome skewing during cellular reprogramming, although the precise mechanism of TERT action in this case is still to be determined.[137] Taken together, the results presented here indicate the importance of proper telomere elongation during derivation of iPS cells and establish telomerase reactivation as a major component of cellular reprogramming.

3.2.2 Derivation of IPS cells harboring disease-associated telomerase mutations

The upregulation of TERT in wild-type mouse and human iPS cells leads to a progressive telomere elongation, resulting in telomeres that are similar in length to telomeres in ES cells.[131,138] In addition, as previously discussed, iPS cells derived from telomerase knockout mouse embryonic fibroblasts show little to none telomere elongation, indicating that telomerase activity is the driving force of telomere elongation and maintenance in iPS cells.[131,137] In human samples, the importance of telomerase activity for telomere maintenance and consequent self-renewal capability of iPS cells can be directly inferred from experiments using cells derived from patients harboring mutations in different components of telomerase and telomere-binding genes, such as DC and aplastic anemia. Since in these diseases, which are discussed in detail elsewhere in this book, patients have varying degrees of telomere attrition and stem cell impairment, the generation of patient-specific iPS cells would represent a unique model to understand the molecular physiology behind telomere shortening induced tissue failure.[139] Accordingly, different research groups tried to pursue this aim and have reported the successful generation of iPS cells derived from DC[140,141] and aplastic anemia patients.[142] Although cellular reprogramming from adult dermal fibroblasts derived from DC patients has proven to be extremely challenging, an optimized protocol where cells are kept and reprogrammed under low-oxygen conditions significantly increased reprogramming efficiency in those samples.[140] Using this improved protocol, the generation of iPS cells from patients carrying different mutations in DKC1, TCAB1, and TERT showed that telomere homeostasis is impaired in telomerase-mutant iPS cells. Surprisingly, it was shown that the stem cell-specific transcription factors OCT4 and NANOG bind to both TERC and dyskerin regulatory elements, thereby increasing their expression,[141] a previously unknown aspect of telomerase regulation in stem cells. Importantly, experiments with these cells also showed the importance of cellular reprogramming for accurate disease modeling *in vitro*. While TERT-mutant iPS cells showed marginal telomere dysfunction, TCAB1- and DKC1-mutant iPS cells presented with a severe telomere dysfunction phenotype, showing progressive telomere shortening that ultimately resulted in the loss of self-renewal of these cells. These results suggest that DC iPS cells might be mimicking the molecular events behind loss of self-renewal in adult stem cell compartments in DC patients, providing an explanation for the recently described correlation between disease severity and telomere length in patients afflicted with this disease.[143] Similar results were obtained from iPS cells derived from aplastic anemia patients, carrying mutations in TERT and TERC, which elongated telomeres at a lower rate when compared with wild-type iPS cells.[142] Importantly, telomerase-mutant iPS cells showed defective hematopoietic differentiation *in vitro*, therefore mimicking the clinical aspects of this disease in patients and demonstrating that human telomere diseases can be accurately modeled utilizing iPS cells.[142] When taken together, these results provide a clear genetic evidence for the importance of telomerase regulation and function in human pluripotent cells.[139]

4. CONCLUSIONS

Telomerase activity is tightly regulated in both mouse and human samples, and the limiting factor for this regulation seems to be TERT expression, which is limited to stem and progenitor cell populations. The importance of telomerase activity for stem cell function is clear in embryonic, adult, and induced pluripotent stem cells, where a failure to properly maintain telomere length leads to loss of self-renewal, the major feature of these cellular populations. The deleterious consequences of telomere-induced loss of self-renewal can be observed in organismal fitness, where it results in reduced tissue function. Additionally, we have discussed the role of TERT in different developmental pathways, independently from its role in telomere elongation. The molecular and biochemical understanding of these process is still in its infancy and is prone to contribute significantly for our knowledge of human health.

REFERENCES

1. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell*. 2008;132(4):567–582.
2. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature*. 1981;292(5819):154–156.

3. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA*. 1981;78(12):7634–7638.
4. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;282(5391):1145–1147.
5. Chen L, Daley GQ. Molecular basis of pluripotency. *Hum Mol Genet*. 2008;17(R1): R23–R27.
6. Young RA. Control of the embryonic stem cell state. *Cell*. 2011;144(6):940–954.
7. Rossant J. Stem cells and early lineage development. *Cell*. 2008;132(4):527–531.
8. Silva J, Smith A. Capturing pluripotency. *Cell*. 2008;132(4):532–536.
9. Snippert HJ, Clevers H. Tracking adult stem cells. *EMBO Rep*. 2011;12(2):113–122.
10. Diehn M, Cho RW, Clarke MF. Therapeutic implications of the cancer stem cell hypothesis. *Semin Radiat Oncol*. 2009;19(2):78–86.
11. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature*. 2010;464(7288):520–528.
12. Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. *Cell*. 1999;97(4):503–514.
13. Karlseder J, Broccoli D, Dai Y, Hardy S, de Lange T. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. *Science*. 1999;283(5406):1321–1325.
14. Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature*. 2007;448(7157):1068–1071.
15. Guo X, Deng Y, Lin Y, et al. Dysfunctional telomeres activate an ATM-ATR-dependent DNA damage response to suppress tumorigenesis. *EMBO J*. 2007;26(22):4709–4719.
16. Hockemeyer D, Palm W, Else T, et al. Telomere protection by mammalian Pot1 requires interaction with Tpp1. *Nat Struct Mol Biol*. 2007;14(8):754–761.
17. Sfeir A, de Lange T. Removal of shelterin reveals the telomere end-protection problem. *Science*. 2012;336(6081):593–597.
18. Giraud-Panis MJ, Teixeira MT, Geli V, Gilson E. CST meets shelterin to keep telomeres in check. *Mol Cell*. 2010;39(5):665–676.
19. Pinto AR, Li H, Nicholls C, Liu JP. Telomere protein complexes and interactions with telomerase in telomere maintenance. *Front Biosci (Landmark Ed)*. 2011;16:187–207.
20. Olovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol*. 1973;41(1):181–190.
21. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature*. 1990;345(6274):458–460.
22. Ballabeni A, Park IH, Zhao R, et al. Cell cycle adaptations of embryonic stem cells. *Proc Natl Acad Sci USA*. 2011;108(48):19252–19257.
23. Fujii-Yamamoto H, Kim JM, Arai K, Masai H. Cell cycle and developmental regulations of replication factors in mouse embryonic stem cells. *J Biol Chem*. 2005;280(13):12976–12987.
24. Ruiz S, Panopoulos AD, Herrerias A, et al. A high proliferation rate is required for cell reprogramming and maintenance of human embryonic stem cell identity. *Curr Biol*. 2011;21(1):45–52.
25. Conklin JF, Baker J, Sage J. The RB family is required for the self-renewal and survival of human embryonic stem cells. *Nat Commun*. 2012;3:1244.
26. Maimets T, Neganova I, Armstrong L, Lako M. Activation of p53 by nutlin leads to rapid differentiation of human embryonic stem cells. *Oncogene*. 2008;27(40):5277–5287.
27. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell*. 1987;51(6):887–898.
28. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. *Cell*. 1985;43(2 Pt 1):405–413.
29. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell*. 1989;59(3):521–529.
30. Niida H, Matsumoto T, Satoh H, et al. Severe growth defect in mouse cells lacking the telomerase RNA component. *Nat Genet*. 1998;19(2):203–206.
31. Wang Y, Erdmann N, Giannone RJ, et al. An increase in telomere sister chromatid exchange in murine embryonic stem cells possessing critically shortened telomeres. *Proc Natl Acad Sci USA*. 2005;102(29):10256–10260.
32. Bailey SM, Brenneman MA, Goodwin EH. Frequent recombination in telomeric DNA may extend the proliferative life of telomerase-negative cells. *Nucleic Acids Res*. 2004;32(12):3743–3751.
33. Niida H, Shinkai Y, Hande MP, et al. Telomere maintenance in telomerase-deficient mouse embryonic stem cells: characterization of an amplified telomeric DNA. *Mol Cell Biol*. 2000;20(11):4115–4127.
34. Zalzman M, Falco G, Sharova LV, et al. Zscan4 regulates telomere elongation and genomic stability in ES cells. *Nature*. 2010;464(7290):858–863.
35. Amano T, Hirata T, Falco G, et al. Zscan4 restores the developmental potency of embryonic stem cells. *Nat Commun*. 2013;4:1966.
36. Huang J, Wang F, Okuka M, et al. Association of telomere length with authentic pluripotency of ES/iPS cells. *Cell Res*. 2011;21(5):779–792.
37. Armstrong L, Saretzki G, Peters H, et al. Overexpression of telomerase confers growth advantage, stress resistance, and enhanced differentiation of ESCs toward the hematopoietic lineage. *Stem Cells*. 2005;23(4):516–529.
38. Yang C, Przyborski S, Cooke MJ, et al. A key role for telomerase reverse transcriptase unit in modulating human embryonic stem cell proliferation, cell cycle dynamics, and in vitro differentiation. *Stem Cells*. 2008;26(4):850–863.
39. Venteicher AS, Abreu EB, Meng Z, et al. A human telomerase holoenzyme protein required for Cajal body localization and telomere synthesis. *Science*. 2009;323(5914):644–648.
40. Blackburn EH, Collins K. Telomerase: an RNP enzyme synthesizes DNA. *Cold Spring Harb Perspect Biol*. 2011;3(5).
41. Mitchell JR, Wood E, Collins K. A telomerase component is defective in the human disease dyskeratosis congenita. *Nature*. 1999;402(6761):551–555.
42. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW. Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet*. 1996;18(2):173–179.
43. Varela E, Schneider RP, Ortega S, Blasco MA. Different telomere-length dynamics at the inner cell mass versus established embryonic stem (ES) cells. *Proc Natl Acad Sci USA*. 2011;108(37):15207–15212.
44. Zeng S, Liu L, Sun Y, et al. Telomerase-mediated telomere elongation from human blastocysts to embryonic stem cells. *J Cell Sci*. 2013;127(Pt 4):752–762.
45. Saretzki G, Walter T, Atkinson S, et al. Downregulation of multiple stress defense mechanisms during differentiation of human embryonic stem cells. *Stem Cells*. 2008;26(2):455–464.
46. Yehezkel S, Rebibo-Sabbah A, Segev Y, et al. Reprogramming of telomeric regions during the generation of human induced pluripotent stem cells and subsequent differentiation into fibroblast-like derivatives. *Epigenetics*. 2011;6(1):63–75.
47. Fuchs E, Chen T. A matter of life and death: self-renewal in stem cells. *EMBO Rep*. 2013;14(1):39–48.
48. Li L, Clevers H. Coexistence of quiescent and active adult stem cells in mammals. *Science*. 2010;327(5965):542–545.
49. Buczacki SJ, Zecchini HI, Nicholson AM, et al. Intestinal label-retaining cells are secretory precursors expressing Lgr5. *Nature*. 2013;495(7439):65–69.

50. Clevers H. Stem cells: a unifying theory for the crypt. *Nature*. 2013;495(7439):53–54.
51. Morrison SJ, Prowse KR, Ho P, Weissman IL. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity*. 1996;5(3):207–216.
52. Yui J, Chiu CP, Lansdorp PM. Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. *Blood*. 1998;91(9):3255–3262.
53. Allsopp RC, Cheshier S, Weissman IL. Telomere shortening accompanies increased cell cycle activity during serial transplantation of hematopoietic stem cells. *J Exp Med*. 2001;193(8):917–924.
54. Vaziri H, Dragowska W, Allsopp RC, et al. Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. *Proc Natl Acad Sci USA*. 1994;91(21):9857–9860.
55. Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL. Telomerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood*. 2003;102(2):517–520.
56. Allsopp RC, Morin GB, Horner JW, et al. Effect of TERT over-expression on the long-term transplantation capacity of hematopoietic stem cells. *Nat Med*. 2003;9(4):369–371.
57. Ramirez RD, Wright WE, Shay JW, Taylor RS. Telomerase activity concentrates in the mitotically active segments of human hair follicles. *J Invest Dermatol*. 1997;108(1):113–117.
58. Ueda M, Ouhtit A, Bito T, et al. Evidence for UV-associated activation of telomerase in human skin. *Cancer Res*. 1997;57(3):370–374.
59. Flores I, Cayuela ML, Blasco MA. Effects of telomerase and telomere length on epidermal stem cell behavior. *Science*. 2005;309(5738):1253–1256.
60. Sarin KY, Cheung P, Gilison D, et al. Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature*. 2005;436(7053):1048–1052.
61. Stange DE, Clevers H. Concise review: the yin and yang of intestinal (cancer) stem cells and their progenitors. *Stem Cells*. 2013;31(11):2287–2295.
62. Leblond CP, Stevens CE. The constant renewal of the intestinal epithelium in the albino rat. *Anat Rec*. 1948;100(3):357–377.
63. Breault DT, Min IM, Carlone DL, et al. Generation of mTert-GFP mice as a model to identify and study tissue progenitor cells. *Proc Natl Acad Sci USA*. 2008;105(30):10420–10425.
64. Montgomery RK, Carlone DL, Richmond CA, et al. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci USA*. 2011;108(1):179–184.
65. Schepers AG, Vries R, van den Born M, van de Wetering M, Clevers H. Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. *EMBO J*. 2011;30(6):1104–1109.
66. Pech MF, Artandi SE. TRAPping telomerase within the intestinal stem cell niche. *EMBO J*. 2011;30(6):986–987.
67. Sacco A, Mourkioti F, Tran R, et al. Short telomeres and stem cell exhaustion model Duchenne muscular dystrophy in mdx/mTR mice. *Cell*. 2010;143(7):1059–1071.
68. Mourkioti F, Kustan J, Kraft P, et al. Role of telomere dysfunction in cardiac failure in Duchenne muscular dystrophy. *Nat Cell Biol*. 2013;15(8):895–904.
69. Gage FH, Temple S. Neural stem cells: generating and regenerating the brain. *Neuron*. 2013;80(3):588–601.
70. Wang S, Chandler-Militello D, Lu G, et al. Prospective identification, isolation, and profiling of a telomerase-expressing subpopulation of human neural stem cells, using sox2 enhancer-directed fluorescence-activated cell sorting. *J Neurosci*. 2010;30(44):14635–14648.

71. Ferron S, Mira H, Franco S, et al. Telomere shortening and chromosomal instability abrogates proliferation of adult but not embryonic neural stem cells. *Development*. 2004;131(16):4059–4070.
72. Eisenberg DT, Hayes MG, Kuzawa CW. Delayed paternal age of reproduction in humans is associated with longer telomeres across two generations of descendants. *Proc Natl Acad Sci USA*. 2012;109(26):10251–10256.
73. Maki CB, Pacchiarotti J, Ramos T, et al. Phenotypic and molecular characterization of spermatogonial stem cells in adult primate testes. *Hum Reprod*. 2009;24(6):1480–1491.
74. Izadyar F, Wong J, Maki C, et al. Identification and characterization of repopulating spermatogonial stem cells from the adult human testis. *Hum Reprod*. 2011;26(6):1296–1306.
75. Yan L, Wu S, Zhang S, Ji G, Gu A. Genetic variants in telomerase reverse transcriptase (TERT) and telomerase-associated protein 1 (TEP1) and the risk of male infertility. *Gene*. 2014;534(2):139–143.
76. Liu L, Bailey SM, Okuka M, et al. Telomere lengthening early in development. *Nat Cell Biol*. 2007;9(12):1436–1441.
77. Schaetzlein S, Lucas-Hahn A, Lemme E, et al. Telomere length is reset during early mammalian embryogenesis. *Proc Natl Acad Sci USA*. 2004;101(21):8034–8038.
78. Blasco MA, Lee HW, Hande MP, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*. 1997;91(1):25–34.
79. Rudolph KL, Chang S, Lee HW, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell*. 1999;96(5):701–712.
80. Lee HW, Blasco MA, Gottlieb GJ, et al. Essential role of mouse telomerase in highly proliferative organs. *Nature*. 1998;392(6676):569–574.
81. Beamer WG, Sweet HO, Bronson RT, et al. Adrenocortical dysplasia: a mouse model system for adrenocortical insufficiency. *J Endocrinol*. 1994;141(1):33–43.
82. Keegan CE, Hutz JE, Else T, et al. Urogenital and caudal dysgenesis in adrenocortical dysplasia (acd) mice is caused by a splicing mutation in a novel telomeric regulator. *Hum Mol Genet*. 2005;14(1):113–123.
83. Hockemeyer D, Palm W, Wang RC, Couto SS, de Lange T. Engineered telomere degradation models dyskeratosis congenita. *Genes Dev*. 2008;22(13):1773–1785.
84. Wang Y, Shen MF, Chang S. Essential roles for Pot1b in HSC self-renewal and survival. *Blood*. 2011;118(23):6068–6077.
85. Savage SA, Alter BP. Dyskeratosis congenita. *Hematol Oncol Clin North Am*. 2009;23(2):215–231.
86. Bessler M, Wilson DB, Mason PJ. Dyskeratosis congenita. *FEBS Lett*. 2010;584(17):3831–3838.
87. Armanios M, Blackburn EH. The telomere syndromes. *Nat Rev Genet*. 2012;13(10):693–704.
88. Armanios M. Syndromes of telomere shortening. *Annu Rev Genomics Hum Genet*. 2009;10:45–61.
89. Imamura S, Uchiyama J, Koshimizu E, et al. A non-canonical function of zebrafish telomerase reverse transcriptase is required for developmental hematopoiesis. *PLoS One*. 2008;3(10):e3364.
90. Choi J, Southworth LK, Sarin KY, et al. TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. *PLoS Genet*. 2008;4(1):e10.
91. Park JI, Venteicher AS, Hong JY, et al. Telomerase modulates Wnt signalling by association with target gene chromatin. *Nature*. 2009;460(7251):66–72.
92. Hoffmeyer K, Raggioli A, Rudloff S, et al. Wnt/beta-catenin signaling regulates telomerase in stem cells and cancer cells. *Science*. 2012;336(6088):1549–1554.

93. Strong MA, Vidal-Cardenas SL, Karim B, et al. Phenotypes in mTERT(+)/(−) and mTERT(−)/(−) mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase. *Mol Cell Biol.* 2011;31(12):2369–2379.
94. Listerman I, Gazzaniga FS, Blackburn EH. An investigation of the effects of the core protein telomerase reverse transcriptase on Wnt signaling in breast cancer cells. *Mol Cell Biol.* 2014;34(2):280–289.
95. Shkreli M, Sarin KY, Pech MF, et al. Reversible cell-cycle entry in adult kidney podocytes through regulated control of telomerase and Wnt signaling. *Nat Med.* 2012;18(1):111–119.
96. Maida Y, Yasukawa M, Furuuchi M, et al. An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature.* 2009;461(7261):230–235.
97. Ridanpaa M, van Eenennaam H, Pelin K, et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage–hair hypoplasia. *Cell.* 2001;104(2):195–203.
98. Hermanns P, Bertuch AA, Bertin TK, et al. Consequences of mutations in the non-coding RMRP RNA in cartilage–hair hypoplasia. *Hum Mol Genet.* 2005;14(23):3723–3740.
99. Rosenbluh J, Nijhawan D, Chen Z, et al. RMRP is a non-coding RNA essential for early murine development. *PLoS One.* 2011;6(10):e26270.
100. Briggs R, King TJ. Transplantation of living nuclei from blastula cells into enucleated frogs' eggs. *Proc Natl Acad Sci USA.* 1952;38(5):455–463.
101. Gurdon JB. The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J Embryol Exp Morphol.* 1962;10:622–640.
102. Gurdon JB, Laskey RA, Reeves OR. The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J Embryol Exp Morphol.* 1975;34(1):93–112.
103. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. *Nature.* 1997;385(6619):810–813.
104. Jaenisch R. Stem cells, pluripotency and nuclear reprogramming. *J Thromb Haemost.* 2009;7(Suppl 1):21–23.
105. Eggan K, Baldwin K, Tackett M, et al. Mice cloned from olfactory sensory neurons. *Nature.* 2004;428(6978):44–49.
106. Hochedlinger K, Jaenisch R. Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature.* 2002;415(6875):1035–1038.
107. Blau HM, Baltimore D. Differentiation requires continuous regulation. *J Cell Biol.* 1991;112(5):781–783.
108. Blau HM, Chiu CP, Webster C. Cytoplasmic activation of human nuclear genes in stable heterocaryons. *Cell.* 1983;32(4):1171–1180.
109. Blau HM, Pavlath GK, Hardeman EC, et al. Plasticity of the differentiated state. *Science.* 1985;230(4727):758–766.
110. Johansson CB, Youssef S, Koleckar K, et al. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. *Nat Cell Biol.* 2008;10(5):575–583.
111. Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. *Proc Natl Acad Sci USA.* 2003;100(4):2088–2093.
112. Yamanaka S, Blau HM. Nuclear reprogramming to a pluripotent state by three approaches. *Nature.* 2010;465(7299):704–712.
113. Shiels PG, Kind AJ, Campbell KH, et al. Analysis of telomere lengths in cloned sheep. *Nature.* 1999;399(6734):316–317.
114. Wakayama T, Shinkai Y, Tamashiro KL, et al. Cloning of mice to six generations. *Nature.* 2000;407(6802):318–319.

115. Lanza RP, Cibelli JB, Blackwell C, et al. Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science.* 2000;288(5466):665–669.
116. Tian XC, Xu J, Yang X. Normal telomere lengths found in cloned cattle. *Nat Genet.* 2000;26(3):272–273.
117. Betts D, Bordignon V, Hill J, et al. Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proc Natl Acad Sci USA.* 2001;98(3):1077–1082.
118. Miyashita N, Kubo Y, Yonai M, et al. Cloned cows with short telomeres deliver healthy offspring with normal-length telomeres. *J Reprod Dev.* 2011;57(5):636–642.
119. Clark AJ, Ferrier P, Aslam S, et al. Proliferative lifespan is conserved after nuclear transfer. *Nat Cell Biol.* 2003;5(6):535–538.
120. Xu J, Yang X. Telomerase activity in early bovine embryos derived from parthenogenetic activation and nuclear transfer. *Biol Reprod.* 2001;64(3):770–774.
121. Dang-Nguyen TQ, Haraguchi S, Akagi S, et al. Telomere elongation during morula-to-blastocyst transition in cloned porcine embryos. *Cell Reprogram.* 2012;14(6):514–519.
122. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007;131(5):861–872.
123. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006;126(4):663–676.
124. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007;318(5858):1917–1920.
125. Warren L, Manos PD, Ahfeldt T, et al. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell.* 2010;7(5):618–630.
126. Anokye-Danso F, Trivedi CM, Juhr D, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell.* 2011;8(4):376–388.
127. Zhou H, Wu S, Joo JY, et al. Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell.* 2009;4(5):381–384.
128. Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. *Nature.* 2012;481(7381):295–305.
129. Stadtfeld M, Maherali N, Breault DT, Hochedlinger K. Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell.* 2008;2(3):230–240.
130. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008;451(7175):141–146.
131. Marion RM, Strati K, Li H, et al. Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell.* 2009;4(2):141–154.
132. Utikal J, Polo JM, Stadtfeld M, et al. Immortalization eliminates a roadblock during cellular reprogramming into iPS cells. *Nature.* 2009;460(7259):1145–1148.
133. Marion RM, Strati K, Li H, et al. A p53-mediated DNA damage response limits reprogramming to ensure iPS cell genomic integrity. *Nature.* 2009;460(7259):1149–1153.
134. Li H, Collado M, Villasante A, et al. The Ink4/Arf locus is a barrier for iPS cell reprogramming. *Nature.* 2009;460(7259):1136–1139.
135. Kawamura T, Suzuki J, Wang YV, et al. Linking the p53 tumour suppressor pathway to somatic cell reprogramming. *Nature.* 2009;460(7259):1140–1144.
136. Hong H, Takahashi K, Ichisaka T, et al. Suppression of induced pluripotent stem cell generation by the p53-p21 pathway. *Nature.* 2009;460(7259):1132–1135.
137. Le R, Kou Z, Jiang Y, et al. Enhanced telomere rejuvenation in pluripotent cells reprogrammed via nuclear transfer relative to induced pluripotent stem cells. *Cell Stem Cell.* 2014;14(1):27–39.

138. Suhr ST, Chang EA, Rodriguez RM, et al. Telomere dynamics in human cells reprogrammed to pluripotency. *PLoS One*. 2009;4(12):e8124.
139. Batista LF, Artandi SE. Understanding telomere diseases through analysis of patient-derived iPS cells. *Curr Opin Genet Dev*. 2013;23(5):526–533.
140. Batista LF, Pech MF, Zhong FL, et al. Telomere shortening and loss of self-renewal in dyskeratosis congenita induced pluripotent stem cells. *Nature*. 2011;474(7351):399–402.
141. Agarwal S, Loh YH, McLoughlin EM, et al. Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. *Nature*. 2010;464(7286):292–296.
142. Winkler T, Hong SG, Decker JE, et al. Defective telomere elongation and hematopoiesis from telomerase-mutant aplastic anemia iPSCs. *J Clin Invest*. 2013;123(5):1952–1963.
143. Alter BP, Rosenberg PS, Giri N, et al. Telomere length is associated with disease severity and declines with age in dyskeratosis congenita. *Haematologica*. 2012;97(3):353–359.

CHAPTER FOUR

Telomere Dynamics and Aging

Geraldine Aubert
Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, British Columbia, Canada

Contents

1. Introduction: Telomere Structure, Determinants of Telomere Dynamics, Measurement Parameters, and Regulation Mechanisms 90
   1.1 About this chapter 90
   1.2 Telomere structure and determinants of telomere dynamics 90
   1.3 Measurement parameters, regulation mechanisms, natural variability, and natural variants 94
2. Telomere Connection to the Characteristics of Aging: Cell Senescence and Apoptosis, Mitochondrial Function, and Metabolism 96
   2.1 Senescence or apoptosis telomere checkpoint 96
   2.2 Telomeres, mitochondrial function, and metabolism 98
3. Telomeres Dynamics in Early Embryonic Development, Stem Cells, and Infancy 99
   3.1 Gametes, embryogenesis, and stem cells 99
   3.2 Inherited telomere length and dynamics in infancy 101
4. Telomere Dynamics and Aging-Related Disorders 102
   4.1 Telomere deficiency syndromes 102
   4.2 Genomic instability and cancer 105
5. Perspective 106
Acknowledgments 106
References 106

Abstract

Telomeres consist of repetitive DNA–protein complexes that cap the ends of vertebrate linear chromosomes. Their capping function and dynamics both with regard to structure and length are carefully orchestrated by many regulatory mechanisms and factors, with likely more yet to be described. Telomere shortening has been shown to be a major measurable molecular characteristic of aging of cells *in vitro* and *in vivo* and is thought to have evolved as a tumor protection mechanism in long-lived species. Regulators and modifiers of telomere dynamics and dynamics with age together with the consequences of telomere shortening and telomere dysfunction in the context of aging and aging-related disorders are discussed in this chapter.

